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Please find below and/or attached an Office communication concerning this application or proceeding.

JL

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/593,914 | HYLDIG-NIELSEN ET AL. | |
| | Examiner | Art Unit | |
| | Carla Myers | 1634 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *01 December 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-8,10-12,16,18,19,21-26,29,32,34,46-49,61,62 and 80-87* is/are pending in the application.

4a) Of the above claim(s) *34* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-12,16,18,19,21-26,29,32,46-49,61,62 and 80-87* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

### *Election/Restrictions*

1. Applicant's election with traverse of Group I, SEQ ID NO: 1 in the reply filed on December 1, 2004 is acknowledged. The traversal is on the ground(s) that the different groupings are not distinct because they are each classified in Class 536, subclass 24.32 and Class 435, subclass 6. Applicants thereby conclude that undue burden would not be required to search the invention of Group I together with the inventions of Groups II-XI. Applicants further assert that the nucleic acids all have the property of being useful for identifying the yeast Dekkera/Brettanomyces and thereby this common feature links the claimed nucleic acid sequences for the purpose of forming a proper Markush group. This is not found persuasive because the classification of a sequence alone is not the determining factor as to whether restriction is proper. In the present case, while the inventions may each have the same classification, the inventions are drawn to distinct nucleic acid sequences, having distinct structural and functional properties. The nucleotide sequence of each of SEQ ID NO: 1-11 (i.e., Groups I-XI) are all distinct from one another. Further, the nucleic acids have different functional properties in that they have distinct melting temperatures and distinct hybridization properties. While each of the sequences can be used to detect species of the yeast Dekkera/Bretanomyces, the sequences differ in their specificities of hybridization and detection. For instance, SEQ ID NO: 1 (PNA probe BRE04) detects D. bruxellensis but this probe does not detect Dekkera anomala. On the other hand, SEQ ID NO: 15 (PNA probe BRE14) detects Dekkera anomala but does not detect D. bruxellensis. The probes each hybridize to

different regions of the genome of Dekkera/Bretanomyces and hybridize with different specificities and melting temperatures and thereby do not in fact share the same structural and functional properties. Further, as set forth in the restriction requirement a search for each of the sequences is not co-extensive. For instance, a search for SEQ ID NO: 1 would not also provide a complete search of SEQ ID NO: 2. Further, if the sequence of SEQ ID NO: 1 is found to be novel and unobvious over the prior art, this would not lead to a holding that SEQ ID NO: 2, for example, is also novel and unobvious over the prior art. Similarly, a reference establishing that SEQ ID NO: 1 was anticipated or rendered obvious over the prior art would not extend to a finding that SEQ ID NO: 2 is also anticipated or rendered obvious over the prior art. Accordingly, it is maintained that a search for each of the distinct inventions would pose a serious burden on the Office. The requirement is still deemed proper and is therefore made FINAL.

Claims 1-8, 10-12, 16, 18, 19, 21-26, 29, 32, 46-49, 61, 62, and 80-87 have been examined herein. Claim 34 is withdrawn from consideration as being drawn to a non-elected invention.

***Claim Rejections - 35 USC § 103***

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-8 and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998).

Kosse discloses dot blot and *in situ* hybridization methods for the detection and enumeration of *Dekkera bruxellensis*. Kosse teaches that the *in situ* hybridization method is performed using fluorescent labeled probes (e.g., TRITC or FLUOS) and that the dot blot hybridization is performed using digoxigenin labeled probes (page 469). The probes are complementary to sequences of the *Dekkera bruxellensis* 18S rRNA region (page 469; Table 2). The reference teaches that prior to *in situ* hybridization, yeast cell walls must be permeabilized and that probes must be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478). Kosse teaches that *Dekkera bruxellensis* was successfully detected by in situ hybridization using 20% formaldehyde (see Table 2 and page 474). Table 2 lists additional 18S rRNA probes for the detection of other yeasts known to cause spoilage of dairy products (see page 468 and Table 2). Probes are also disclosed which are specific for all yeasts and for all eukaryotes (Table 2). Kosse further teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products and other foods so as to ensure high quality and safe food products (see page 468).

Kosse teaches that the *in situ* hybridization method is performed using fluorescent- labeled probes and that dot blot hybridization is performed using digoxigenin labeled probes. Kosse does not specifically teach using enzyme-linked probes, or specifically soy bean peroxidase labeled probes.

However, Stender (1998) teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Kosse so as to have specifically labeled the probes with soy bean peroxidase in order to have achieved the benefit of providing an effective means for labeling the probes, thereby facilitating the detection of *Dekkera bruxellensis.*

With respect to claim 4, Kosse does not teach using PNA probes. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms. Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of ordinary skill in the art at the time the

invention was made to have modified the probes of Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

**Response to arguments:**

In the response, Applicants state that the claims are limited to enzyme-linked in situ hybridization probes. Applicants state that the rejection is not proper and that no motivation or reasonable expectation of success has been provided for applying the enzyme-linked in situ probe technology to the detection of yeasts. Applicants assert that the rejection merely takes elements from various references and combines them using the specification as a blueprint to achieve the description of the claimed subject matter.

Applicant's arguments have been fully considered but are not persuasive to overcome the present grounds of rejection. With respect to Applicants assertion that no motivation has been provided to combine the references, the test of obviousness under 35 U.S.C. 103 is not express suggestion of the claimed invention in any or all of the references but what references taken collectively would suggest to those of ordinary skill in the art presumed to be familiar with them (In re Rosselet, 146 USPQ 183(CCPA 1965). For the purposes of combining references, those references need not explicitly suggest combining teachings much less specific references, In re Nilseen, 851 F. 2d 1401, 7 USPQ2d 1500 (Fed Cir. 1988). As stated in Ex parte Levengood, 28 USPQ2d 1300, "In order to establish a *prima facie* case of obviousness, it is necessary for the

examiner to present *evidence*, preferably in the form of some teaching, suggestion, incentive or inference in the applied prior art, or in the form of generally available knowledge, that one having ordinary skill in the art would have been led to combine the relevant teachings of the applied references in the proposed manner to arrive at the claimed invention." The motivation for combining the teachings of the various references need not be explicitly found in the references themselves, but may be provided by the examiner based on logic and sound scientific reasoning. In the instant case, Stender teaches that enzyme labels may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). Accordingly, the teachings in the art when considered as a whole provide the motivation to modify the probes of Kosse so as to have labeled the probes with an enzyme label such as soy bean peroxidase because this would have provided an equally effective means for labeling the probes and for facilitating the detection of hybridization between the probes and their target sequence.

Applicants further assert that the teachings of Amann indicate that one would not have had a reasonable expectation of success at using yeast enzyme linked probes for in situ hybridization. However, Applicants have not accurately characterized the teachings of Amann. There are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts. Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae. Amann does not teach that these results apply to the detection of all yeasts. While Amann teaches that an enzyme-labeled probe was not useful for the detection of

Saccharomyces cerevisiae, the reference teaches that modifying the conditions for permeabilization of cells allows one to use enzyme-labeled probes for some organisms. The specification provides no teachings as to the critical steps that must be performed in order to allow for the detection of yeasts by in-situ hybridization using enzyme-labeled probes and the claims clearly do not recite any critical steps which distinguish the claims over prior art *in-situ* hybridization methods of detecting microorganisms using enzyme-labeled probes. Accordingly, given the teachings in the art of methods for detecting microorganisms using enzyme-labeled probes and the knowledge in the art of how to modify the conditions of permeabilization in order to allow for larger molecules to penetrate cells, the ordinary artisan would have had a reasonable expectation of success of applying the *in-situ* hybridization methods of Stender to the detection of yeast using enzyme labeled probes.

Further, the teachings of Stender indicate that it would have been obvious to have labeled any probe with an enzyme. Kosse teaches the use of probes for performing the method of in situ hybridization probes and the method of dot blot hybridization. As set forth in the rejection, it would have also been obvious to one of ordinary skill in the art at the time the invention was made to have labeled the probes to be used for dot blot hybridization with an enzyme label because this would have provided an equally effective means for labeling the probes and detecting nucleic acid hybridized to said probes. Applicants response concentrates on their position that it is unobvious to label probes that are used in *in situ* hybridization assays. However, the response does not address the use of enzymes to label probes for non-in situ

hybridization assays assays. The recitation in the claims of "An enzyme-linked in-situ hybridization probe" constitutes an intended use of the probe and does not distinguish the probes over those of Kosse. There are no structural limitations in the claims which define what constitutes an in situ hybridization probe and what distinguishes in situ hybridization probes over other types of hybridization probes. The recitation of "in situ hybridization probe" does not impart any particular structural limitation. As such, the claims encompass any enzyme-labeled probe that hybridizes to a yeast target sequence, including the dot blot hybridization probes of Kosse labeled with an enzyme, as taught by Stender.

Applicants further assert that the combined art is not analogous. It is asserted that Stender is directed to mycobacteria and not to yeast and thereby does not constitute analogous art. This argument has been fully considered but is not persuasive. Both the Kosse and Stender reference are directed to methods of dot blot and in situ hybridization and the labeling of hybridization probes. Thereby, the Stender reference does in fact represent analogous art. Analogous art does not require that the art is identical to the primary reference or teaches the same subject matter as the primary reference. Those of ordinary skill in the art would recognize that the labeling of probes that contain sequences to Mycobacteria would be analogous to the labeling of probes which contain sequences of yeast. Applicants have not established that there is a distinction between the methodology for labeling mycobacteria probes with enzyme moieties and the methodology for labeling yeast probes with enzyme moieties.

Applicants argue that the 132 Declaration of Dr. Stender supports the position that "there simply cannot be any expectation of success in combining Kosse and Stender." Applicants assert that the declaration establishes that Amann teaches away from the claimed invention. Applicants arguments and the 132 Declaration of Dr. Stender have been fully considered but are not persuasive to overcome the present grounds of rejection. The Declaration states that "Stender (1998) would not, in view of Amann tend to motivate the application of enzyme-linked probes to in-situ assays for yeast because the reference does not address yeast." However, this is a statement of opinion by one of the present inventors and this statement is not supported by any scientific evidence or arguments. Secondly, the statement is based only on how the present inventor believes the ordinary artisan would view the teachings of Stender and Kosse in view of the Amann 1992 reference. The statement does not take into consideration the teachings in the art as a whole.

The Declaration sates that based upon review of the Amann reference, the declarant believe that "1) Amann et al teach that it was well accepted, at the time of their publication, that enzyme-linked (labeled) probes COULD NOT readily penetrate the cell wall of yeast; 2) Amann et al. had no success with getting enzyme-labeled probes into yeast; and 3) Amann et al. would tend to dissuade one of skill in the art from attempting to use an enzyme-linked probe to analysis a yeast in an in-situ based assay." Amann 1992 establishes that some experimentation is required to obtain enzyme-labeled in situ hybridization probes. Such a teaching does not teach away from the claimed invention. Obviousness does not require absolute predictability but only the

reasonable expectation of success. See In re Merck and Company Inc., 800 F. 2d 1091, 231 USPQ 375 (Fed. Cir. 1986) and In re O'Farrell, 7 USPQ2d 1673 (Fed. Cir. 1988). In the present case, the teachings and guidance provided in the art indicate that it is within the skill of the art to obtain enzyme yeast-labeled probes by practicing routine experimentation. The experimentation required to obtain and use yeast enzyme-labeled in situ hybridization probes does not appear to be inventive, but rather appears to be ordinary. Applicants do not provide any evidence or arguments to show that undue experimentation was required to arrive at the present invention. Rather, Applicants followed the teachings already disclosed in the art in order to arrive at the present invention. While all probes for yeast may not be effective for in situ hybridization purposes, the selection of those probes that are effective can be accomplished by routine experimentation. Again, there are no teachings in the specification as to what modifications in the methodology of the prior art were required to arrive at the claimed invention. Further, if the field of obtaining a yeast enzyme labeled in situ hybridization probe is in fact that unpredictable, then the showing in the specification of 11 probes for Dekkera species would clearly not be sufficient to support a claim to a genus to any enzyme-linked *in situ* hybridization probe to any species or genus of yeast. There are no teachings in Applicant's specification which would allow one of skill in the art to achieve results that are an improvement over those achieved by Amann when applied to in situ hybridization using enzyme labeled probes for any yeast. Applicants do not teach any particular conditions of permeabilization, conditions for in situ hybridization or manner

for selecting probes which would allow one to extend their findings with 11 Dekkera probes to all other yeasts.

Further, Applicants response asserts that Amann 1992 teaches away from the claimed invention. However, Applicants response does not acknowledge the teachings of Amann 1995 (note the filing date of the present invention is 6/14/2000). Amann et al. (Microbiological Reviews. March 1995. 59(1): 143-169) provides extensive guidance as to how to select a probe for in situ hybridization and provides more than a reasonable expectation that the ordinary artisan could obtain probes to be used to detect yeast by in situ hybridzation. Amann (page 158-166) addresses several criteria which may effect in situ hybridization efficiency and provides guidance as to how to improve the specificity and sensitivity of in situ hybridization. Amann acknowledges that some regions of the rRNA are less accessible for hybridization to probes due to the presence of secondary structures in the rRNA (see pages 159-160). Amann outlines the steps for evaluating the ability of a probe to effectively hybridize to the rRNA in situ and teaches how to optimize probes with respect to selecting an appropriately accessible target sequence, selecting optimal probes and hybridization conditions based on the probe's temperatures of dissociation, and labeling probes with enzymes (page 160). At page 160, Amann states that "There is some indication (although from negative results) that certain regions might be inaccessible in certain phylogenetic groups. Sometimes, shifting the target site by just a few nucleotides has a major influence on the probe sensitivity." Lastly, it is again pointed out that the Amann 1992 reference (page 3010) states that: "We are continuing to evaluate alternative approaches to permeabilize

whole fixed cells. Although we are optimistic that methods can be tailored for single strains under investigation, we do not expect to find a universal method that will permeabilize the whole array of different microorganisms to a comparable degree. This means that general probes (e.g., for the three domains) should not be used as enzyme derivatives for the characterization of environmental samples because they will likely produce biased results. Nevertheless, highly specific enzyme-probe conjugates used in combination with suitable methods to permeabilize specific cells of interest should provide a valuable tool for the detection and identification of individual cells in situ." Given these teachings of Amann, it is maintained that it was within the skill of the art at the time the invention was made to have identified conditions appropriate for the use of in situ hybridization methods in yeasts. Amann teaches that the conditions of permeabilization must be optimized in order to allow one to use enzyme-labeled probes in in situ hybridization methods in yeast cells. Applicants have not provided any evidence to establish that undue experimentation would be required to identify such conditions. Nor are such conditions set forth in the claims or disclosed in the specification in a manner which would make clear that such conditions are applicable to all species of yeast.

3. Claims 47-49 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of Kosse and Stender are presented above. The combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, Kosse and Stender do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean

peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

**Response to arguments:**

In the response, Applicants traverse this rejection for the same reasons above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

4. Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 46, 61-62, 86 and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and further in view of Stender et al (1998; reference BB).

De Wachter teaches an isolated nucleic acid consisting of the sequence of the 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter is considered to have the property of being suitable as a probe for the detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*. De Wachter does not teach labeling the 18S rRNA with a detectable moiety, and particularly does not teach labeling the probe with an enzyme.

Kosse teaches hybridization methods, including dot blot hybridization and in situ hybridization, for the detection of *Dekkera bruxellensis*. Kosse teaches labeling probes with either chemiluminescent labels (e.g., digoxigenin) or fluorescent labels (e.g., TRITC

or FLUOS) to facilitate the detection of yeasts and to particularly facilitate the detection of *Dekkera bruxellensis* (see page 469). The reference further exemplifies an 18S rRNA probe specific for *Dekkera bruxellensis* (see Table 2) and probes specific for other yoghurt spoiling yeasts. Kosse teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products, such as yoghurt, so as to ensure high quality and safe food products (see page 468).

In view of the teachings of Kosse, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have used the labeled 18S rRNA of De Wachter as a probe under suitable hybridization conditions in order to have facilitated the detection of *Dekkera bruxellensis* in dairy samples. Additionally, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have generated probe sets comprising one or more probes for *Dekkera bruxellensis* or comprising probes for *Dekkera bruxellensis* and probes for other yeast and to have labeled each probe with a different detectable moiety in order to have allowed for the detection and differentiation of multiple types of yeast in dairy products, such as yoghurt.

The combined teachings of De Wachter and Kosse do not teach labeling the probes with soy bean peroxidase. However, Stender teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter in view of

Kosse by using soy bean peroxidase to label the probes in order to have provided an equally effective probe for the detection of *Dekkera bruxellensis.*

Secondly, De Wachter and Kosse do not teach immobilizing the *D. bruxellensis* and/or yeast probes onto a solid support. However, Stender teaches that hybridization probes may be immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of De Wachter and Kosse onto a solid support, as taught by Stender, in order to have achieved the benefit of simultaneously assaying for the presence of target sequences complementary to a multitude of different probes.

Thirdly, the combined references do not teach PNA probes for the detection of *Dekkera bruxellensis*. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms. Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of

ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter and Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

With respect to claim 25, De Wachter and Kosse do not teach adding blocking probes to the probe sets. Stender (page 25, 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of De Wachter and Kosse so as to have included the "blocking probes" disclosed by Stender in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

**Response to arguments:**

In the response, Applicants traverse this rejection by stating that Kosse teaches that only the 3' end of the 18S rRNA is accessible to fluorescently labeled probes and that other variable regions are not accessible to in-situ hybridization. Applicants conclude that it is not reasonable to expect that any nucleotide which is homologous to the gene sequence described by De Wachter can be used to produce in situ hybridization probes.

Applicants arguments have been fully considered but are not persuasive. Firstly, it is noted that while claims 1-9 are limited to in situ hybridization probes and claim 46 is limited to a method of in situ hybridization, the remaining claims are not limited to in situ hybridization probes or to methods of in situ hybridization. Accordingly, Applicants are

arguing limitations that are not recited in claims 10-12, 16, 18-19, 21-26, 29, 32, 61-62, 86 and 87. All of the probes disclosed by Kosse were found to be useful for dot blot hybridization, regardless of whether they were derived from the 5' end or 3' end of the rRNA. Secondly, Applicants claims recite the language of "comprising" and thereby include the 5' sequences of the rRNA. Thus, Applicants claims are not limited to the subject matter which provides the asserted unexpected results. Additionally, there are no teachings in Applicants specification which indicate that the probes should consist of only the 3' end of the rRNA and no guidance provided in the specification which would indicate that the 5' end of the rRNA should be excluded from the hybridization probes. Thirdly, Kosse teaches that some regions of the 5' 18S rRNA may not be useful as species-specific probes because the target region may not be accessible for hybridization. However, Kosse does not provide any comments regarding probes which include both the 5' end and 3' end of the 18S rRNA. Thereby, Applicants have not established that the probe of De Wachter labeled with an enzyme moiety would not be suitable for in-situ hybridization or other types of hybridization.

Additionally, it is again pointed out that each of Applicants arguments is directed to the use of probes for in situ hybridization. Claims 10-12, 16, 18-19, 21-26, 29, 32, 61-62, 86 and 87 are not directed to in situ hybridization probes. Further, as discussed above, claims 1-8 do not recite any limitations which distinguish an "in situ hybridization probe" from a "dot blot hybridization probe." As such, the recitation in the preamble of "in situ hybridization probe" merely states the purpose or intended use of the invention.

This recitation does not distinguish the probes over the hybridization probes of Kosse labeled with an enzyme moiety.

Applicants point out that Amann states that "For now, enzyme-labeled oligonucleotides can therefore be used only for specific detection of gram-negative bacteria and several members of Euryarchaeota." Thereby, Applicants conclude that Amann had no success in using enzyme labeled probes for such analysis. Applicants state that the teachings of Amann amount to "wishful thinking and bald speculation." It is also stated that "More importantly, since 1992, it appears that Amann and his colleagues have not been successful in the asserted endeavor." Applicants assert that "little weight should be accorded given to the conclusory statements of Amann." Applicants arguments regarding the Amann 1992 reference have been addressed fully above. In summary, Applicants arguments and the Declaration of Dr. Stender do not establish that undue experimentation would be required for the ordinary artisan to have generated the claimed. The experimentation required to arrive at the claimed invention would have been well within the level and skill of the ordinary artisan given the guidance provided by Amann 1995 and 1992. Further, Applicants arguments regarding unexpected results are not commensurate in scope with the breadth of the claims.

5. Claims 47-49 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of De Wachter, Kosse and Stender are presented above. The combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, the combined references do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of De Wachter, Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation

solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

**Response to arguments:**

In the response, Applicants traverse this rejection for the same reasons stated in above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (571) 272-0747. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571)-272-0745.

The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

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Carla Myers
March 7, 2005

CARLA J. MYERS
PRIMARY EXAMINER